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## BIOASSAY DATA FOR MARINE POLLUTION USING SEA URCHIN EGGS, 1972 AND 1973

### 1972

Ten experiments for biological assay were made using sea urchin eggs to check marine pollution around the Seto Marine Biological Laboratory.

I. Winter season, January 16. Eggs of *Hemicentrotus pulcherrimus* (A. Agassiz) were used, see Table 1.

II. Spring season, three experiments were made in March-May using *Hemicentrotus pulcherrimus* or *Anthocardaris crassispina* (A. Agassiz) eggs.

1. March 2, see Table 2.
2. April 1, see Table 3.
3. May 14, see Table 4.

III. Summer season, four experiments were made in June-August, using *Anthocardaris crassispina* eggs.

1. June 11, see Table 5.
2. July 11, see Table 6.
3. August 7, see Table 7.
4. August 24, see Table 8.

IV. Autumn season, two experiments were made in September-November, using *Anthocardaris crassispina* or *Pseudocentrotus depressus* (A. Agassiz) eggs.

1. September 23, see Table 9.
2. November 23, see Table 10.

(Notes common to all tables: Fertilization membrane formation examined 3 min. after insemination; minutes and hours in parentheses respectively after First cleavage and Gastrulation indicate the time after insemination; the maturation state of gonads used was nearly ripe to full ripe; Degree of inhibitory effect 1 shows the non-inhibitory and ordinary state and 2 the weakly inhibitory state of the sea water (see Kobayashi 1972).)

The COD values were measured by Dr. Sh. Fuse.

### 1973

Seven experiments were made as follows.

I. Winter season, February 21, Eggs of *Hemicentrotus pulcherrimus* were used, see Table 11.

II. Spring season, two experiments were made in March-May, using *Hemicentrotus pulcherrimus* or *Anthocardaris crassispina* eggs.

1. March 20, see Table 12.
2. May 4, see Table 13.

III. Summer season, two experiments were made in July-August, using *Anthocardaris crassispina* eggs.

1. July 3, see Table 14.
2. August 30, see Table 15.

IV. Autumn season, two experiments were made in September-November, using *Anthocardaris crassispina* or *Pseudocentrotus depressus* eggs.

1. September 14, see Table 16.
2. November 27, see Table 17.

(Notes common to all tables: See the notes mentioned above, besides the following ones; For the 0 hr. old eggs, Degree of inhibitory effect 1 shows the non-inhibitory and ordinary state and 2 the weakly inhibitory state of the sea water (see Publ. Seto Mar. Biol. Lab., Vol. XIX, No. 6, p. 378, Table 2, 1972. For the 3-10 hrs. old eggs, Degree of inhibitory effect 0 shows the non-inhibition state, 1 the slight inhibition, 2 the weak inhibition and 3 the moderate inhibition state of the sea water (see Publ. Seto Mar. Biol. Lab., Vol. XXI, No. 5/6, p. 391 Table 8, 1974).

The COD values were measured by Dr. Sh. Fuse.

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Table 1. Results of the Jan. 16 experiment with eggs of *Hemicentrotus pulcherrimus*.  
Wind: NW 2. Test water temperature: 17°C. (warmed).

Location	Fertiliz.	First cleavage (90 min.)			Gastrulation (24 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polysperm)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running sea water of Laboratory	99.6%	0.8%	99.2%	0%	0.5%	99.5%	0%		ppm	1
	99.2	1.0	99.0	0	0.3	99.7	0			
	99.7	0.7	99.3	0	0.2	99.8	0			
Water from open sea side of Hatakejima Surface	99.5	0.9	99.1	0	0.3	99.7	0			1
	99.4	0.7	99.2	0.1	0.2	99.8	0			
	99.5	1.0	99.0	0	0.2	99.7	0.1			
Water from land side of Hatakejima Surface	99.4	1.0	99.0	0	0.2	99.8	0			1
	99.3	1.1	98.9	0	0.7	99.3	0			
	99.6	0.5	99.4	0.1	0.6	99.4	0			
Sea water from Tsunashirazu cove Surface	99.3	0.9	99.1	0	0.3	99.6	0.1			1
	99.4	1.0	99.0	0	0.6	99.4	0			
	99.2	1.0	98.9	0.1	0.7	99.2	0.1			

Table 2. Results of the Mar. 2 experiment with eggs of *Hemicentrotus pulcherrimus*.  
Wind: NW 1. Test water temperature: 18°C. (warmed).

Location	Fertiliz.	First cleavage (90 min.)			Gastrulation (24 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polysperm)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running sea water of Laboratory	99.8%	0.8%	99.2%	0 %	0.2%	99.8%	0 %		ppm	1
	98.3	2.1	97.7	0.2	0.2	99.8	0			
	99.1	0.7	99.2	0.1	0.1	99.9	0			
Water from open sea side of Hatakejima Surface	99.7	0.7	99.3	0	0.1	99.9	0			1
	98.2	2.2	97.4	0.4	0.2	99.7	0.1			
	99.2	0.5	99.3	0.2	0.2	99.8	0			
Water from land side of Hatakejima Surface	99.3	1.3	98.5	0.2	0.2	99.8	0			1
	98.1	2.5	97.2	0.3	0.4	99.5	0.1			
	99.0	1.8	97.9	0.3	0.3	99.6	0.1			
Sea water from Tsunashirazu cove Surface	98.7	1.9	97.7	0.4	0.3	99.6	0.1			1
	97.2	2.9	96.7	0.4	0.4	99.6	0			
	98.7	2.3	97.4	0.3	0.3	99.7	0			

Table 3. Results of the Apr. 1 experiment with eggs of *Hemicentrotus pulcherrimus*.  
Wind. NW 2. Test water temperature: 18°C. (warmed).

Location	Fertiliz.	First cleavage (90 min.)			Gastrulation (24 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polysperm)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running sea water of Laboratory	97.8%	3.2%	96.8%	0 %	3.2%	96.7%	0.1%		ppm	1
	98.3	2.2	97.8	0	0.7	99.3	0			
	96.8	4.7	95.3	0	2.9	97.0	0.1			
Water from open sea side of Hatakejima Surface	87.9	12.7	87.3	0	4.3	95.5	0.2			1
	90.2	10.2	89.4	0.4	0.9	99.1	0			
	89.3	12.8	87.2	0	3.1	96.7	0.2			
Water from land side of Hatakejima Surface	97.1	4.2	95.8	0	3.3	96.5	0.2			1
	97.3	4.6	93.4	0	0.9	99.0	0.1			
	95.2	5.7	94.3	0	3.2	96.6	0.2			
Sea water from Tsunashirazu cove Surface	84.2	16.4	83.6	0	4.7	95.0	0.3			1
	85.3	14.8	84.9	0.3	1.1	98.8	0.1			
	83.3	17.5	82.5	0	3.2	96.5	0.3			

Table 4. Results of the May 14 experiment with eggs of *Anthocidaris crassispina*.  
Wind: 0. Test water temperature: 20°C.

Location	Fertiliz.	First cleavage (75 min.)			Gastrulation (24 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polysperm)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running sea water of Laboratory	98.1%	2.7%	97.3%	0 %	7.3%	92.4%	0.3%		ppm	1
	99.2	1.7	98.3	0	0.7	99.3	0		1.96	
	98.3	1.2	98.1	0.7	0.6	99.4	0			
Water from open sea side of Hatakejima Surface	97.8	3.0	97.0	0	7.3	92.6	0.1			1
	98.7	1.6	98.4	0	1.0	99.0	0		2.20	
	97.6	1.6	97.2	1.2	0.8	99.2	0			
Water from land side of Hatakejima Surface	94.3	5.6	92.3	2.1	7.8	91.8	0.4			1
	96.3	2.7	95.7	1.6	2.8	97.1	0.1		3.12	
	97.1	1.7	96.8	1.5	0.2	99.0	0			
Sea water from Tsunashirazu cove Surface	91.3	6.7	90.1	3.2	8.3	91.2	0.5			1
	92.3	4.6	93.1	2.3	3.3	96.5	0.2		2.90	
	95.1	5.0	92.3	2.7	2.7	97.3	0			

Table 5. Results of the June 11 experiment with eggs of *Anthocidaris crassispina*.  
Wind: 0. Test water temperature: 23°C.

Location	Fertiliz.	First cleavage (75 min.)			Gastrulation (20 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polysperm)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running sea water of Laboratory	99.4%	0.6%	99.3%	0.1%	0.6%	99.4%	0 %		ppm	1
	98.7	1.4	98.5	0.1	2.3	97.5	0.2		1.66	
	99.2	0.9	99.0	0.1	1.2	98.7	0.1			
Water from open sea side of Hatakejima Surface	99.5	0.5	99.3	0.2	0.7	99.3	0			1
	98.8	1.3	98.5	0.2	3.1	96.6	0.3		1.96	
	99.3	0.9	99.1	0	1.4	98.5	0.1			
Water from land side of Hatakejima Surface	99.3	0.7	99.1	0.2	0.9	99.1	0			1
	98.5	1.4	98.3	0.3	3.3	96.5	0.2		1.70	
	99.1	1.1	98.8	0.1	2.3	97.6	0.1			
Sea water from Tsunashirazu cove Surface	98.9	1.1	98.7	0.2	1.3	98.7	0			1
	98.1	2.2	97.5	0.3	3.8	95.9	0.3		2.08	
	98.4	1.9	97.9	0.2	3.4	96.4	0.2			

Table 6. Results of the July 11 experiment with eggs of *Anthocidaris crassispina*.  
Wind: S 2. Test water temperature: 26°C.

Location	Fertiliz.	First cleavage (60 min.)			Gastrulation (17 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polysperm)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running sea water of Laboratory	92.5%	7.6%	92.4%	0 %	0.2%	99.8%	0 %		ppm	1
	94.5	5.7	94.3	0	0.1	99.9	0		0.16	
	93.2	7.0	93.0	0	0.3	99.7	0			
Water from open sea side of Hatakejima Surface	93.4	6.8	93.2	0	0.1	99.9	0			1
	95.2	5.0	95.0	0	0.1	99.9	0		0.89	
	93.1	7.1	92.8	0.1	0.2	99.8	0			
Water from land side of Hatakejima Surface	92.1	8.2	91.8	0	0.3	99.7	0			1
	94.1	6.3	93.7	0	0.1	99.9	0		1.18	
	93.3	6.9	93.1	0	0.4	99.6	0			
Sea water from Tsunashirazu cove Surface	91.3	8.9	91.0	0.1	0.4	99.6	0			1
	93.8	6.4	93.6	0	0.2	99.8	0		0.52	
	92.5	7.5	92.4	0.1	0.4	99.6	0			

Table 7. Results of the Aug. 7 experiment with eggs of *Anthocidaris crassipina*.  
Wind NNE 1. Test water temperature: 28°C.

Location (depth)	Fertiliz.	First cleavage (50 min.)			Gastrulation (15 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polysperm)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	97.3%	3.8%	96.2%	0 %	0.6%	99.4%	0 %		ppm	1
	93.8	8.1	91.5	0.4	0.5	99.5	0			
	97.8	3.2	96.8	0	0.5	99.5	0			
Water from open sea side fo Hatakejima Surface	98.2	3.4	96.6	0	0.7	99.3	0			1
	94.2	6.3	93.7	0	0.8	99.2	0			
	96.3	4.1	95.9	0	0.6	99.4	0			
Bottom (25)	94.5	12.4	87.6	0	1.1	98.9	0			1
	89.3	13.5	86.2	0.3	1.3	98.7	0			
	91.3	9.7	90.3	0	0.9	99.1	0			
Water from land side of Hatakejima Surface	93.4	11.3	88.7	0	0.8	99.2	0			1
	87.3	15.3	84.7	0	0.9	99.1	0			
	89.8	12.7	87.3	0	0.8	99.2	0			
Bottom (27)	92.3	15.6	84.4	0	1.3	98.7	0			2
	84.3	18.9	80.4	0.7	1.5	98.5	0			
	87.5	16.0	83.7	0.3	1.2	98.8	0			
Sea water from Tsunashirazu cove Surface	87.5	17.8	82.2	0	6.3	93.7	0	slightly		2
	89.8	12.1	87.3	0.6	18.3	81.7	0	delay		
	84.9	19.3	80.4	0.3	12.9	87.1	0			
Bottom (5)	83.2	21.3	78.0	0.7	5.3	94.7	0	slightly		2
	81.3	19.5	79.9	0.6	7.2	92.8	0	delay		
	78.2	24.3	74.9	0.8	8.3	91.7	0			

Table 8. Results of the Aug. 24 experiment with eggs of *Anthocardis crassispina*.  
Wind: 0. Test water temperature: 25°C.

Location (depth)	Fertiliz.	First cleavage (60 min.)			Gastrulation (17 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polysperm)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	99.3%	0.9%	98.3%	0.8%	0.7%	99.3%	0 %		ppm	1
	98.3	1.2	98.1	0.7	0.8	99.2	0			
	96.9	3.7	96.2	0.1	0.8	99.1	0.1			
Water from open sea side of Hatakejima Surface	99.4	0.8	98.9	0.3	0.9	99.1	0			1
	99.0	1.1	98.1	0.8	0.9	99.1	0			
	96.8	3.5	96.3	0.2	1.0	99.0	0			
Bottom (25)	99.3	0.8	98.7	0.5	1.1	98.9	0			1
	98.9	1.2	97.9	0.9	1.4	98.6	0			
	96.8	3.6	96.1	0.3	1.7	98.3	0			
Water from land side of Hatakejima Surface	92.2	9.0	90.1	0.9	1.9	98.1	0			1
	91.7	8.5	90.4	1.1	2.4	97.6	0			
	89.2	13.5	86.0	0.5	2.5	97.5	0			
Bottom (27)	88.5	11.8	87.4	0.8	2.7	97.3	0			1
	88.3	12.0	86.8	1.2	2.5	97.5	0			
	86.0	13.6	85.8	0.6	4.5	95.5	0			
Sea water from Tsunashirazu cove Surface	88.3	11.7	87.5	0.8	2.9	97.1	0			1
	88.7	11.6	87.4	1.0	3.5	96.5	0			
	86.2	13.4	86.1	0.5	3.9	96.1	0			
Bottom (5)	87.9	12.3	86.5	1.2	2.7	97.3	0			1
	87.6	12.5	86.2	1.3	3.8	96.2	0			
	85.3	13.7	85.6	0.7	5.7	94.3	0			

Table 9. Results of the Sep. 23 experiment with eggs of *Anthocidaris crassispina*.  
Wind: E 1. Test water temperature: 26°C.

Location (depth)	Fertiliz.	First cleavage (60 min.)			Gastrulation (17 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polysperm)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running sea water of Laboratory (m)	91.5%	11.5%	88.5%	0 %	0.5%	99.5%	0 %		ppm 1.37	1
	87.5	15.0	85.0	0	1.0	99.0	0			
Water from open sea side of Hatakejima Surface	86.5	14.5	85.5	0	2.0	98.0	0		1.96	1
	85.5	17.0	83.0	0	2.0	98.0	0			
Bottom (25)	86.0	16.0	84.0	0	2.0	98.0	0		1.81	1
	85.0	17.5	82.5	0	3.5	96.5	0			
Water from land side of Hatakejima Surface	85.0	19.5	80.5	0	2.0	98.0	0		2.04	1
	85.0	20.0	80.0	0	2.5	97.5	0			
Bottom (27)	84.5	20.0	80.0	0	2.5	97.5	0		2.18	1
	83.5	21.0	79.0	0	3.5	96.5	0			
Sea water from Tsunashirazu cove Surface	85.5	19.5	80.5	0	2.5	97.5	0		2.29	1
	85.0	20.0	80.0	0	3.0	97.0	0			
Bottom (5)	83.0	21.0	79.0	0	3.5	96.5	0		1.51	2
	82.5	22.5	77.5	0	4.0	96.0	0			



Table 10. Results of the Nov. 23 experiment with eggs of *Pseudocentrotus depressus*.  
Wind: NW 1. Test water temperature: 14°C.

Location (depth)	Fertiliz.	First cleavage (120 min.)			Gastrulation (24 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polysperm)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running sea water of Laboratory (m)	98.3%	0.8%	98.0%	1.2%	0.2%	99.8%	0 %		ppm 0.81	1
	99.7	0.4	99.6	0	0.1	99.9	0			
Water from open sea side of Hatakejima Surface	98.5	1.1	98.2	0.7	0.3	99.7	0		1.08	1
	99.5	0.6	99.2	0.2	0.2	99.8	0			
Bottom (25)	96.9	2.4	96.3	1.3	0.3	99.7	0		0.85	1
	99.6	0.4	99.4	0.2	0.2	99.8	0			
Water from land side of Hatakejima Surface	96.7	1.8	96.5	1.7	0.4	99.6	0		0.61	1
	99.5	0.3	99.4	0.3	0.1	99.9	0			
Bottom (27)	96.5	2.3	95.8	1.9	0.3	99.7	0		1.16	1
	99.2	0.6	99.0	0.4	0.2	99.8	0			
Sea water from Tsunashirazu cove Surface	96.3	2.4	95.5	2.1	0.4	99.6	0		1.29	1
	99.3	0.4	99.2	0.4	0.2	99.8	0			
Bottom (5)	96.2	2.6	95.0	2.4	0.5	99.5	0		1.07	1
	99.3	0.7	98.9	0.4	0.3	99.7	0			

Table 11. Results of the Feb. 21 experiment with eggs of *Hemicentrotus pulcherrimus*.  
Wind; NW 1. Test water temperature; 17°C (warmed). 0 hr. old eggs

Location (depth)	Fertiliz.	First cleavage (90 min.)			Gastrulation (24 hrs.)			Other notes		Degree of inhibitory effect I
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	99.5%	1.0%	99.0%	0 %	0.5%	99.5%	0 %		ppm 1.07	1
	99.0	2.0	98.0	0	0.5	99.5	0			
	97.5	3.5	96.5	0	0	100	0			
Water from open sea side of Hatakejima Surface	98.5	2.0	98.0	0	1.0	99.0	0			
	97.5	2.5	97.5	0	1.5	98.5	0		1.09	1
	99.0	1.0	99.0	0	0.5	99.5	0			
Bottom (25)	98.0	2.5	97.5	0	1.0	99.0	0			
	96.5	3.0	97.0	0	1.0	99.0	0		0.84	1
	99.5	4.0	96.0	0	1.5	98.5	0			
Water from land side of Hatakejima Surface	98.0	3.0	97.0	0	1.5	98.5	0			
	97.0	4.0	96.0	0	1.0	99.0	0		1.13	1
	97.5	4.5	95.5	0	2.5	97.5	0			
Bottom (27)	97.0	7.5	92.5	0	2.5	97.5	0			
	95.0	5.5	94.5	0	1.5	98.5	0		1.33	1
	93.0	9.0	91.0	0	3.0	97.0	0			
Sea water from Tsunashirazu cove Surface	93.5	16.5	83.5	0	2.0	98.0	0			
	94.0	14.5	85.5	0	3.0	97.0	0		1.30	1
	91.5	12.0	88.0	0	4.5	95.5	0			
Bottom (5)	94.5	15.5	84.5	0	4.0	96.0	0			
	93.5	17.0	83.0	0	4.5	95.5	0		1.01	1
	93.0	15.5	84.5	0	3.0	97.0	0			

Table 11. (continued).

7 hrs. old eggs

Location (depth)	Fertiliz.	First cleavage (90 min.)			Gastrulation (24 hrs.)			Other notes		Degree of inhibitory effect II
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	99.0%	2.0%	98.0	0 %	0.5%	99.5%	0 %		ppm	0
	98.5	3.0	97.0	0	1.0	99.0	0			
	96.0	5.0	95.0	0	0.5	99.5	0			
Water from open sea side of Hatakejima Surface	94.5	6.0	93.5	0.5	1.0	99.0	0			0
	93.5	7.5	92.5	0	1.0	99.0	0			
	93.0	8.5	91.5	0	0.5	99.5	0			
Bottom (25)	96.0	8.5	91.5	0	0.5	99.5	0			0
	94.0	8.0	92.0	0	1.0	99.0	0			
	93.5	7.0	93.0	0	1.0	99.0	0			
Water from land side of Hatakejima Surface	92.0	23.0	77.0	0	5.5	94.5	0			2
	90.5	25.0	75.0	0	6.5	93.5	0			
	89.5	23.5	76.5	0	6.0	94.0	0			
Bottom (27)	93.5	20.5	79.5	0	8.0	92.0	0			2
	89.0	25.5	74.5	0	8.5	91.5	0			
	87.5	24.0	76.0	0	6.5	93.5	0			
sea water from Tsunashirazu cove Surface	87.0	28.0	72.0	0	7.5	92.5	0			2
	83.5	29.5	70.5	0	8.5	91.5	0			
	84.0	29.0	71.0	0	7.0	93.0	0			
Bottom (5)	91.0	26.5	72.5	1.0	8.5	91.5	0			2
	89.5	26.0	73.5	0.5	7.5	92.5	0			
	87.5	28.0	71.0	1.0	8.5	91.5	0			

Table 12. Results of the Mar. 20 experiment with eggs of *Hemicentrotus pulcherrimus*.  
Wind; 0. Test water temperature; 15°C.

0 hr. old eggs

Location (depth)	Fertiliz.	First cleavage (150 min.)			Gastrulation (32 hrs.)			Other notes		Degree of inhibitory effect I
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	99.5%	1.0%	98.5%	0.5%	0.5%	99.5%	0 %		ppm	1
	100	0	100	0	1.0	99.0	0			
	97.0	4.0	96.0	0	0.5	99.5	0			
Water from open sea side of Hatakejima Surface	99.5	1.0	99.0	0	1.0	99.0	0			1
	99.5	2.0	98.0	0	0	100	0			
	98.0	3.0	97.0	0	0.5	99.5	0			
Bottom (25)	97.5	4.5	95.5	0	1.0	99.0	0			1
	100	2.0	98.0	0	0.5	99.5	0			
	94.0	8.0	92.0	0	1.0	99.0	0			
Water from land side of Hatakejima Surface	98.0	2.5	97.5	0	1.0	99.0	0			1
	99.0	1.5	98.5	0	1.0	99.0	0			
	95.0	9.0	91.0	0	1.5	98.5	0			
Bottom (27)	96.5	4.5	94.0	1.5	1.0	99.0	0			1
	98.0	2.5	97.5	0	1.5	98.5	0			
	95.5	8.5	91.5	0	2.0	98.0	0			
Sea water from Tsunashirazu cove Surface	96.0	6.5	93.5	0	1.0	99.0	0			1
	98.0	5.0	95.0	0	2.0	98.0	0			
	95.0	8.0	92.0	0	2.0	98.0	0			
Bottom (5)	96.5	4.0	96.0	0	1.5	98.5	0			1
	97.5	3.5	96.5	0	2.0	98.0	0			
	96.0	13.0	87.0	0	2.0	98.0	0			

Table 12. (continued).

7 hrs. old eggs

Location (depth)	Fertiliz.	First cleavage (150 min.)			Gastrulation (32 hrs.)			Other notes		Degree of inhibitory effect II
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	94.0%	8.0%	90.0%	2.0	0.5%	99.5%	0 %		ppm	1
	95.5	6.0	93.0	1.0	0.5	99.5	0			
	93.0	9.0	91.0	0	1.0	99.0	0			
Water from open sea side of Hatakejima Surface	92.5	10.5	88.5	1.0	1.0	99.0	0			1
	94.5	8.0	91.5	0.5	1.5	98.5	0			
	91.0	11.0	89.0	0	1.0	99.0	0			
Bottom (25)	91.0	13.5	84.5	2.0	2.0	98.0	0			1
	92.5	13.0	86.0	1.0	2.5	97.5	0			
	90.0	15.0	85.0	0	2.5	97.5	0			
Water from land side of Hatakejima Surface	91.5	18.5	80.0	1.5	4.5	95.5	0			2
	92.5	16.5	82.5	1.0	5.5	94.5	0			
	88.5	20.5	79.0	0.5	4.0	96.0	0			
Bottom (27)	89.5	21.0	78.0	1.0	5.0	95.0	0			2
	90.5	19.0	80.0	1.0	5.0	95.0	0			
	88.0	24.0	76.0	0	5.5	94.5	0			
Sea water from Tsunashirazu cove Surface	88.0	29.5	69.5	1.0	7.5	92.5	0			3
	89.5	27.0	72.5	0.5	8.0	92.0	0			
	85.5	30.5	69.5	0	7.0	93.0	0			
Bottom (5)	87.5	24.5	74.0	1.5	8.0	92.0	0			3
	89.0	29.0	70.0	1.0	9.5	90.5	0			
	82.5	30.5	69.5	0	11.0	89.0	0			

Table 13. Results of the May 4 experiment with eggs of *Anthocidaris crassispina*.  
Wind; 0. Test water temperature; 16°C.

0 hr. old eggs

Location (depth)	Fertiliz.	First cleavage (120 min.)			Gastrulation (30 hrs.)			Other notes		Degree of inhibitory effect I
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	93.5%	8.0%	92.0%	0 %	1.0%	99.0%	0 %		ppm	1
	98.5	3.0	97.0	0	0.5	99.5	0		0.66	
	99.0	2.0	97.5	0.5	0.5	99.5	0			
Water from open sea side of Hatakejima Surface	94.5	7.0	93.0	0	0.5	99.5	0		0.81	1
	96.5	5.5	94.5	0	0	100	0			
	97.5	4.0	96.0	0	0.5	99.5	0			
Bottom (25)	92.0	8.5	91.5	0	1.0	99.0	0		1.11	1
	97.0	4.5	95.5	0	0.5	99.5	0			
	95.5	6.0	94.0	0	0.5	99.5	0			
Water from land side of Hatakejima Surface	91.0	10.0	89.5	0.5	1.0	99.0	0		1.65	1
	90.0	10.5	88.0	1.5	1.5	98.5	0			
	93.0	8.5	91.5	0	1.5	98.5	0			
Bottom (27)	89.5	13.0	87.0	0	1.5	98.5	0		2.09	1
	90.5	11.0	89.0	0	1.5	98.5	0			
	91.5	11.0	89.0	0	2.0	98.0	0			
Sea water from Tsunashirazu cove Surface	87.0	16.0	84.0	0	2.0	98.0	0		0.72	1
	88.5	14.0	86.0	0	2.5	97.5	0			
	87.5	14.5	85.5	0	2.0	98.0	0			
Bottom (5)	86.5	15.5	84.5	0	2.0	98.0	0		1.05	1
	89.0	14.5	85.5	0	2.0	98.0	0			
	90.0	14.0	86.0	0	2.5	97.5	0			

Table 13. (continued).

6 hrs. old eggs

Location (depth)	Fertiliz.	First cleavage (120 min.)			Gastrulation (30 hrs.)			Other notes		Degree of inhibitory effect II
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	92.5%	8.5%	91.5%	0 %	1.0%	99.0%	0 %		ppm	0
	94.0	7.0	92.0	1.0	0.5	99.5	0			
	94.5	6.5	93.0	0.5	0.5	99.5	0			
Water from open sea side of Hatakejima Surface	94.0	9.0	91.0	0	0.5	99.5	0			0
	96.0	7.0	92.5	0.5	0.5	99.5	0			
	97.5	5.0	95.0	0	0	100	0			
Bottom (25)	90.5	10.5	89.5	0	1.0	99.0	0			1
	93.0	9.5	90.5	0	0.5	99.5	0			
	94.5	9.0	91.0	0	0.5	99.5	0			
Water from land side of Hatakejima Surface	85.5	17.5	82.5	0	2.0	98.0	0			1
	90.0	11.5	87.5	1.0	2.5	97.5	0			
	91.5	11.0	88.5	0.5	1.5	98.5	0			
Bottom (27)	84.5	17.5	82.5	0	3.5	96.5	0			1
	87.0	14.5	85.0	0.5	3.5	96.5	0			
	89.0	13.5	86.5	0	2.5	97.5	0			
Sea water from Tsunashirazu cove Surface	81.0	21.5	78.5	0	4.5	95.5	0			2
	84.0	18.5	80.5	1.0	4.0	96.0	0			
	85.0	18.5	81.5	0	3.0	97.0	0			
Bottom (5)	83.5	19.5	80.5	0	4.0	96.0	0			2
	87.0	15.0	84.0	1.0	3.5	96.5	0			
	89.5	14.0	85.5	0.5	2.0	98.0	0			

Table 14. Results of the July 3 experiment with eggs of *Anthocardaris crassispina*.  
Wind; 0. Test water temperature; 26°C.

0 hr. old eggs

Location (depth)	Fertiliz.	First cleavage (60 min.)			Gastrulation (17 hrs.)			Other notes		Degree of inhibitory effect I
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	96.5%	5.0%	95.0%	0 %	1.0%	99.0%	0 %		ppm	1
	97.0	5.5	94.5	0	0.5	99.5	0		0.95	
	97.5	5.0	95.0	0	0.5	99.5	0			
Water from open sea side of Hatakejima Surface	95.5	7.0	93.0	0	1.5	98.5	0		1.33	1
	97.5	4.5	95.5	0	1.5	98.5	0			
	98.0	3.5	96.5	0	1.0	99.0	0			
Bottom (25)	96.0	6.5	93.5	0	1.0	99.0	0		2.53	1
	96.5	4.5	95.5	0	1.5	98.5	0			
	96.0	4.5	95.5	0	1.0	99.0	0			
Water from land side of Hatakejima Surface	93.5	17.5	82.5	0	2.0	98.0	0		0.71	1
	92.5	18.5	81.5	0	1.5	98.5	0			
	93.5	18.0	82.0	0	1.5	98.5	0			
Bottom (27)	93.0	19.5	80.5	0	2.5	97.5	0		2.02	1
	93.5	17.5	82.5	0	2.0	98.0	0			
	93.5	18.0	82.0	0	2.0	98.0	0			
Sea water from Tsunashirazu cove Surface	89.5	22.5	77.5	0	3.0	97.0	0		2.64	2
	88.5	24.0	76.0	0	3.0	97.0	0			
	89.0	23.0	77.0	0	2.5	97.5	0			
Bottom (5)	90.5	22.5	77.5	0	3.5	96.5	0		2.42	2
	91.0	20.5	79.5	0	3.0	97.0	0			
	89.5	23.5	76.5	0	4.0	96.0	0			



Table 14. (continued).

3 hrs. old eggs

Location (depth)	Fertiliz.	First cleavage (60 min.)			Gastrulation (17 hrs.)			Other notes		Degree of inhibitory effect II
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	90.5%	10.0%	90.0%	0 %	1.0%	99.0%	0 %		ppm	1
	89.5	11.5	88.5	0	1.0	99.0	0			
	89.5	10.5	89.5	0	2.0	98.0	0			
Water from open sea side of Hatakejima Surface	91.5	9.5	90.5	0	1.5	98.5	0			1
	90.5	10.0	90.0	0	2.0	98.0	0			
	89.5	13.0	87.0	0	2.5	97.5	0			
Bottom (25)	92.0	9.0	91.0	0	1.0	99.0	0			1
	91.0	10.0	90.0	0	1.5	98.5	0			
	88.5	12.5	87.5	0	2.5	97.5	0			
Water from land side of Hatakejima Surface	87.5	24.0	76.0	0	2.5	97.5	0			2
	89.5	20.5	79.5	0	2.0	98.0	0			
	84.5	25.0	75.0	0	3.5	96.5	0			
Bottom (27)	85.0	26.5	73.5	0	2.5	97.5	0			2
	85.5	22.0	78.0	0	2.5	97.5	0			
	83.5	25.5	74.5	0	3.0	97.0	0			
Sea water from Tsunashirazu cove Surface	83.5	29.5	70.5	0	3.0	97.0	0			3
	84.5	32.0	68.0	0	3.5	96.5	0			
	82.5	31.0	69.0	0	4.5	95.5	0			
Bottom (5)	82.5	28.5	71.5	0	3.5	96.5	0			3
	81.5	31.5	68.5	0	4.0	96.0	0			
	80.0	29.0	71.0	0	4.0	96.0	0			

Table 15. Results of the Aug. 30 experiment with eggs of *Anthocidaris crassispina*.  
Wind; 0. Test water temperature; 28°C.

0. hr. old eggs

Location (depth)	Fertiliz.	First cleavage (50 min.)			Gastrulation (12 hrs.)			Other notes		Degree of inhibitory effect I
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	97.5%	3.5%	96.5%	0 %	1.0%	99.0%	0 %		ppm	1
	96.5	4.0	96.0	0	0.5	99.5	0		0.87	
	97.0	3.0	97.0	0	0.5	99.5	0			
Water from open sea side of Hatakejima Surface	98.0	2.5	97.5	0	1.0	99.0	0			1
	97.5	3.0	97.0	0	1.0	99.0	0		1.22	
	96.5	4.0	96.0	0	0.5	99.5	0			
Bottom (25)	98.0	2.5	97.5	0	1.0	99.0	0			1
	97.0	3.0	97.0	0	2.0	98.0	0		1.45	
	96.5	4.5	95.5	0	1.5	98.5	0			
Water from land side of Hatakejima Surface	96.0	13.5	86.5	0	1.5	98.5	0			1
	96.5	15.5	84.5	0	2.0	98.0	0		1.80	
	95.5	15.5	84.5	0	2.0	98.0	0			
Bottom (27)	94.5	14.5	85.5	0	2.0	98.0	0			1
	95.0	17.0	83.0	0	2.5	97.5	0		2.67	
	94.0	18.5	81.5	0	2.5	97.5	0			
Sea water from Tsunashirazu cove Surface	84.0	20.5	79.5	0	3.0	97.0	0			2
	88.5	19.5	80.5	0	3.0	97.0	0		1.24	
	86.5	21.5	78.5	0	3.5	96.5	0			
Bottom (5)	85.5	21.0	79.0	0	3.5	96.5	0			2
	87.0	21.5	78.5	0	4.0	96.0	0		3.04	
	87.0	21.0	79.0	0	4.5	95.5	0			

Table 15. (continued).

3 hrs. old eggs

Location (depth)	Fertiliz. membrane formation	First cleavage (50 min.)			Gastrulation (12 hrs.)			Other notes		Degree of inhibitory effect II
		1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	93.5%	7.5%	92.5%	0 %	1.0%	99.0%	0 %		ppm	0
	94.5	7.0	93.0	0	1.0	99.0	0			
	92.0	9.0	91.0	0	1.5	98.5	0			
Water from open sea side of Hatakejima Surface	92.5	7.5	92.0	0.5	1.5	98.5	0			0
	93.0	8.0	91.5	0.5	1.0	99.0	0			
	91.5	9.5	90.5	0	1.5	98.5	0			
Bottom (25)	91.0	9.5	90.0	0.5	1.5	98.5	0			1
	90.0	11.0	89.0	0	1.5	98.5	0			
	90.0	11.0	88.0	1.0	2.0	98.0	0			
Water from land side of Hatakejima Surface	89.5	12.0	87.0	1.0	2.0	98.0	0			2
	87.0	13.0	85.5	1.5	2.5	97.5	0			
	86.5	19.5	79.5	1.0	2.5	97.5	0			
Bottom (27)	86.5	14.5	84.0	1.5	3.5	96.5	0			2
	84.0	17.0	81.0	2.0	3.0	97.0	0			
	82.0	18.5	79.5	2.0	3.5	96.5	0			
Sea water from Tsunashirazu cove Surface	82.0	15.5	73.5	1.0	4.5	95.5	0			3
	80.5	16.5	71.0	2.5	4.0	96.0	0			
	76.5	28.5	69.5	2.0	4.0	96.0	0			
Bottom (5)	82.5	26.0	72.5	1.5	4.5	95.5	0			3
	75.0	26.0	71.5	2.5	5.0	95.0	0			
	72.5	28.5	69.0	2.5	5.0	95.0	0			

Table 16. Results of the Sept. 14 experiment with eggs of *Anthocidaris crassispina*.  
Wind NW 1. Test water temperature; 25°C.

0 hr. old eggs

Location (depth)	Fertiliz.	First cleavage (60 min.)			Gastrulation (17 hrs.)			Other notes		Degree of inhibitory effect I
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	98.5%	2.5%	97.5%	0 %	0.5%	99.5%	0 %		ppm	1
	98.5	2.0	98.0	0	1.0	99.0	0			
	97.5	4.0	96.0	0	1.5	98.5	0			
Water from open sea side of Hatakejima Surface	99.0	1.5	98.5	0	0.5	99.5	0			1
	99.5	1.0	99.0	0	1.5	98.5	0			
	95.5	6.0	94.0	0	5.5	94.5	0			
Bottom (25)	98.5	2.0	98.0	0	0.5	99.5	0			1
	99.0	1.5	98.5	0	0.5	99.5	0			
	97.0	4.0	96.0	0	1.0	99.0	0			
Water from land side of Hatakejima Surface	87.5	13.5	86.5	0	1.5	98.5	0			1
	89.0	12.5	87.5	0	3.0	97.0	0			
	84.0	17.0	83.0	0	3.5	96.5	0			
Bottom (27)	84.5	17.0	83.0	0	2.5	97.5	0			2
	85.0	16.0	84.0	0	3.5	96.5	0			
	80.0	21.5	78.5	0	1.5	98.5	0			
Sea water from Tsunashirazu cove Surface	81.0	19.5	80.5	0	3.0	97.0	0			2
	83.0	18.0	82.0	0	2.0	98.0	0			
	80.5	21.0	79.0	0	1.5	98.5	0			
Bottom (5)	80.0	22.0	78.0	0	2.5	97.5	0			2
	83.0	20.5	79.5	0	2.0	98.0	0			
	79.0	22.0	78.0	0	3.5	96.5	0			

Table 16. (continued).

3 hrs. old eggs

Location (depth)	Fertiliz.	First cleavage (60 min.)			Gastrulation (17 hrs.)			Other notes		Degree of inhibitory effect II
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running (m) sea water of Laboratory	97.5%	3.0%	97.0%	0 %	1.0%	99.0%	0 %		ppm	0
	98.5	2.5	97.5	0	0.5	99.5	0			
	96.5	4.0	96.0	0	1.5	98.5	0			
Water from open sea side of Hatakejima Surface	98.0	2.5	97.5	0	1.0	99.0	0			0
	98.5	2.0	98.0	0	0.5	99.5	0			
	97.0	3.0	97.0	0	4.0	96.0	0			
Bottom (25)	97.5	3.0	97.0	0	2.0	98.0	0			0
	99.0	1.5	98.5	0	1.5	98.5	0			
	97.0	3.5	96.5	0	3.0	97.0	0			
Water from land side of Hatakejima Surface	85.5	17.0	83.0	0	1.5	98.5	0			1
	87.5	13.5	86.5	0	2.0	98.0	0			
	83.0	17.5	82.5	0	2.5	97.5	0			
Bottom (27)	73.5	30.5	69.5	0	6.5	93.5	0			3
	72.5	29.5	70.5	0	6.0	94.0	0			
	70.5	33.0	67.0	0	7.0	93.0	0			
Sea water from Tsunashirazu cove Surface	77.0	26.0	74.0	0	5.5	94.5	0			2
	75.0	25.5	74.5	0	6.5	93.5	0			
	73.5	27.0	73.0	0	7.0	93.0	0			
Bottom (5)	75.5	31.0	69.0	0	9.5	90.5	0			3
	74.5	28.0	72.0	0	6.5	93.5	0			
	69.0	33.0	67.0	0	8.0	92.0	0			

Table 17. Results of the Nov. 27 experiment with eggs of *Pseudocentrotus depressus*.  
Wind; 0. Test water temperature; 13°C.

0 hr. old eggs

Location (depth)	Fertiliz.	First cleavage (150 min.)			Gastrulation (35 hrs.)			Other notes		Degree of inhibitory effect
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastula (normal)	exogastrula	abnormal develop.	COD	
Running sea water of Laboratory (m)	98.5%	2.5%	97.5%	0 %	1.0%	99.0%	0 %		ppm	1
	99.5	1.0	99.0	0	0.5	99.5	0			
Water from open sea side of Hatakejima Surface	98.5	2.5	97.5	0	1.0	99.0	0			1
	98.0	3.0	97.0	0	0.5	99.5	0			
Bottom (25)	98.0	3.0	97.0	0	1.5	99.5	0			1
	97.0	3.5	96.5	0	1.0	99.0	0			
Water from land side of Hatakejima Surface	97.0	4.5	95.5	0	1.5	98.5	0			1
	97.0	4.0	96.0	0	2.0	98.0	0			
Bottom (27)	93.0	8.5	91.5	0	2.5	97.5	0			1
	91.5	10.0	90.0	0	2.0	98.0	0			
Sea water from Tsunashirazu cove Surface	89.5	13.0	87.0	0	3.0	97.0	0			1
	87.5	14.0	86.0	0	2.0	98.0	0			
Bottom (5)	84.0	18.5	81.5	0	3.5	96.5	0			2
	85.0	16.5	83.5	0	3.0	97.0	0			

Table 17. (continued).

10 hrs. old eggs

Location (depth)	Fertiliz.	First cleavage (150 min.)			Gastrulation (35 hrs.)			Other notes		Degree of inhibitory effect II
	membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exogastrula	abnormal develop.	COD	
Running sea water of Laboratory (m)	93.5%	9.0%	91.0%	0 %	0.5%	99.5%	0 %		ppm	0
	94.5	7.5	92.5	0	1.0	99.0	0			
Water from open sea side of Hatakejima Surface	94.0	7.0	93.0	0	2.0	98.0	0			0
	94.5	6.0	94.0	0	1.0	99.0	0			
Bottom (25)	92.5	9.5	90.5	0	1.5	98.5	0			0
	93.0	8.0	92.0	0	2.0	98.0	0			
Water from land side of Hatakejima Surface	90.5	14.0	86.0	0	1.5	98.5	0			1
	92.0	10.0	90.0	0	2.0	98.0	0			
Bottom (27)	87.0	18.0	81.5	0.5	2.5	97.5	0			2
	84.5	19.5	80.0	0.5	3.0	97.0	0			
Sea water from Tsunashirazu cove Surface	90.0	16.5	83.5	0	3.5	96.5	0			1
	93.0	10.5	89.5	0	3.5	96.5	0			
Bottom (5)	81.5	21.0	78.0	1.0	3.5	96.5	0			2
	80.5	21.0	78.5	0.5	4.5	95.5	0			